

What is claimed is:

1. A site specific recombination method for
5 removal of predetermined nucleic acid sequences from the
plastid genome, said method comprising:

a) providing a first nucleic acid construct,
said construct comprising a promoter being operably
linked to a nucleic acid encoding an optional plastid
10 targeting transit sequence which is operably linked to a
nucleic acid encoding a protein having excision
activity, said construct further comprising a first
selectable marker encoding nucleic acid having plant
specific 5' and 3' regulatory nucleic acid sequences;

15 b) providing a second DNA construct, said
second construct comprising a second selectable marker
encoding nucleic acid and excision sites, said second
construct optionally containing a gene of interest, said
second construct further comprising flanking plastid
20 targeting nucleic acid sequences which facilitate
homologous recombination into said plastid genome;

c) introducing said second DNA construct into
a plant cell;

25 d) culturing said plant cell of step c) in the
presence of a selection agent, thereby selecting for
those plant cells expressing the proteins encoded by
said second DNA construct;

30 e) introducing said first DNA construct into
plant cells from step d) in the presence of a selection
agent and selecting those plant cells expressing
proteins encoded by said first construct, which when
present said excising activity acts on said excision
sites, thereby excising said predetermined target
sequence.

2. A method as claimed in claim 1, wherein a plant is regenerated from plant cells of step c), cells are then contacted with said first construct and steps d) and e) are performed.

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3. A method as claimed in claim 1, wherein said first construct is that depicted in Figure 3.

10 4. A method as claimed in claim 1, wherein said second construct is that depicted in Figure 2.

15 5. A method as claimed in claim 1, wherein said protein having excision activity is selected from the group consisting of CRE, flippase, resolvase, FLP, SSV1-encoded integrase, and transposase.

6. A method as claimed in claim 1, wherein said excision sites are LOX sequences, and frt sequences.

20 7. A method as claimed in claim 1, wherein said selection agent is selected from the group consisting of kanamycin, gentamycin, spectinomycin, streptomycin and hygromycin, phosphinotricin, basta, glyphosate and bromoxynil.

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8. A method as claimed in claim 1, wherein said excision of said predetermined sequence creates an expressible translational fusion protein.

30 10. A method as claimed in claim 1, wherein said predetermined target sequence is the selectable marker encoding nucleic acid present in said second construct.

11. A plant regenerated from the method of claim 1.

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12. A site specific recombination system comprising the constructs of claim 1.

5 13. A site specific recombination method for removal of predetermined nucleic acid sequences from the plastid genome, said method comprising:

10 a) providing a first nucleic acid construct, said construct comprising a regulated promoter being operably linked to a nucleic acid encoding an optional plastid targeting transit sequence which is operably linked to a nucleic acid encoding a protein having excision activity, said construct optionally further comprising a first selectable marker encoding nucleic acid having plant specific 5' and 3' regulatory nucleic acid sequences;

15 b) providing a second DNA construct, said second construct comprising an second selectable marker encoding nucleic acid and excision sites, said second construct further comprising flanking plastid targeting nucleic acid sequences which facilitate homologous recombination into said plastid genome at a predetermined target sequence such that excision sites flank said predetermined target sequence following homologous recombination;

20 c) introducing said second DNA construct into a plant cell;

25 d) culturing a plant cell of step c) in the presence of a selection agent, thereby selecting for those plant cells expressing the proteins encoded by said second DNA construct;

30 e) regenerating a plant from cells obtained in step d);

35 f) introducing said first DNA construct into plant cells from step e) in the presence of a selection agent and selecting those plant cells expressing

proteins encoded by said first construct, which when present said excising activity acts on said excision sites, thereby excising said predetermined target sequence.

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14. A method as claimed in claim 13, wherein said regulatable promoter is selected from the group of promoters consisting of inducible promoters, tissue specific promoters, developmentally regulated promoters 10 and chemically inducible promoters.

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15. A method as claimed in claim 13, wherein said predetermined target sequence is selected from the group consisting of genes associated with male sterility, clpP ribosomal proteins, ribosomal RNA operon sequences.

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16. A method as claimed in claim 13, wherein said protein having excision activity is selected from the group consisting of CRE, flippase, resolvase, FLP, SSV1-20 encoded integrase, and transposase.

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17. A method as claimed in claim 13, wherein said excision sites are LOX sequences, and frt sequences.

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18. A method as claimed in claim 13, wherein said selection agent is selected from the group consisting of kanamycin, gentamycin, spectinomycin, streptomycin and hygromycin, phosphinotricin, basta, glyphosate and bromoxynil.

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19. A plant regenerated from the method of claim 13.

20. A site specific recombination system for removal of predetermined nucleic acid sequences comprising the construct of claim 13.

5 21. Progeny plants obtained from the plant of claim 11.

22. Progeny plants obtained from the plant of claim 18.

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